BIOTECHNOLOGY

Pick 'n' mix for pest control

Although chemicals that are produced naturally by microorganisms can be used for pest control, modified versions of these compounds often prove to be more effective. A group from Japan has found a way of combining bacterial genes with a fungal host to enhance the properties of an anthelmintic agent.

PF1022A is a cyclodepsipeptide produced by a filamentous fungus of the *Rosellinia* genus that has biological activity against helminthic crop pests. PF1022A activity can be increased by adding amino and nitro groups to its benzene rings, but synthetic methods of doing this involve using large quantities of nitric acid, which is expensive, dangerous and bad for the environment. Koji Yanai and colleagues devised a method to genetically manipulate *Rosellinia* to express alternative substrates for PF1022A biosynthetic enzymes, allowing amino and nitro derivatives to be produced biologically.

D-phenyllactate is one of the substrates of PFSYN, the main enzyme in PF1022A production, and is made in the *Rosellinia* phenylpyruvate biosynthetic pathway. PFSYN also recognizes the related compounds *p*-nitro- and *p*-amino-D-phenyllactate to produce the desired nitro and amino PF1022A derivatives, but these alternative PFSYN substrates are not naturally produced in *Rosellinia*. They are, however, synthesized in the *p*-amino-phenylpyruvate pathway of the bacterium *Streptomyces venezuelae*. So, introducing *S. venezuelae p*-amino-phenylpyruvate biosynthetic genes into *Rosellinia* could enable the production of the more potent PF1022A-related compounds.

To do this, Yanai and colleagues blocked endogenous *Rosellinia* phenylpyruvate synthesis by disrupting the *cmu1* gene, which encodes an enzyme required for the initial step in the pathway. They then isolated the *S. venezuelae* genes required for *p*-amino-phenylpyruvate production — *papA*, *papB* and *papC*. Plasmids carrying these genes were used to transform the *cmu1*-deficient *Rosellinia* strain, and transformants carrying all three genes were selected for and grown in fermentation medium. Analysis of the compounds present in the growth medium of these transformants by mass spectrometry and NMR confirmed that the desired PF1022A derivatives — and other related compounds — were successfully produced.

So, could this method be used for the large-scale production of anthelmintic agents? The amounts obtained in the study by Yanai and colleagues were far too small to be used industrially. However, the authors suggest that yields could be significantly increased by modifying the PFSYN enzyme to use the artificially introduced substrates more efficiently. If this can be done successfully, this 'mix and match' method combining bacterial and fungal genes could provide a feasible and environmentally friendly means of producing agents for pest control.

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References and links

ORIGINAL RESEARCH PAPER Yanai, K. *et al.* *Para*-position derivatives of fungal anthelmintic cyclodepsipeptides engineered with *Streptomyces venezuelae* antibiotic biosynthetic genes. *Nature Biotechnol.* **22**, 848–855 (2004)